

HYDRODYNAMIC FACILITATION AND CONSTRAINT OF DISPERSAL BETWEEN ISOLATED HABITATS

Final Report for AASERT Award N00014-95-I-0763
For the period 01 June 1995 to 31 May 1998

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Long-Term Goals:

The long-term goals of this research program are to understand the processes that control the dispersal, gene flow and diversity of benthic organisms living in patchy, isolated habitats. We address these questions through a variety of approaches, ranging from field studies of larval dispersal to laboratory studies of molecular phylogeny. The study focused originally on seamount habitats, but has been expanded to a wide range of coastal environments including coral reefs and island shores to solve broader questions of phylogeny and evolution of corals, anemones and other anthozoans.

Scientific Objective:

The specific goals of this AASERT project are to resolve two particular phylogenetic questions within the Class Anthozoa (Phylum Cnidaria) using direct sequencing techniques. Traditional, morphology-based, systematic studies of these groups have resulted in substantial controversy over their phylogenetic relationships and evolution. Our first question addresses relationships within the Subclass Octocorallia; traditional ordinal divisions based on morphological traits indicate that seven distinct groups exist within the Octocorallia, but critics argue that this is an overestimate of genetic divergence. The second question addresses the controversy of whether two or three subclasses exist within the Class Anthozoa. By resolving these questions, we can determine which of the suite of morphological characters currently used by taxonomists are truly phylogenetically relevant. We can also infer which characters (and taxa) are ancestral and which are more recently evolved. This project comprises the thesis research of Ewann Berntson (né J. Ewann Agenbroad), a student in the WHOI/MIT Joint Program, and also supported research by Scott France (WHOI Postdoctoral Scholar who is now an Assistant Professor in Biology at College of Charleston)

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These phylogenetic objectives have diverged substantially from the original aim of using molecular studies to evaluate gene flow and dispersal of benthic species inhabiting isolated seamounts. We deviated from this original aim for two reasons: 1) the two segments of the genome that we were using (mitochondrial 16S rRNA and nuclear 18S rRNA) were not sufficiently variable for population-level studies; and 2) we realized that the molecular results provided a unique opportunity to address important phylogenetic questions.

Approach:

The complete 18S rRNA sequence for representative species from each anthozoan family was generated through a sequential procedure of PCR, TA-cloning and sequencing on a Licor automated sequencer. Some of these specimens were acquired fresh via submersible, SCUBA and intertidal collecting, but many others had to be obtained from museum collections. These dried and ethanol-preserved specimens required special procedures for DNA extraction, amplification and sequencing. Phylogenetic relationships were evaluated using maximum likelihood analyses because less sophisticated algorithms for genetic relatedness and distance (e.g., Kimura 2-parameter, Jin/Nei, and parsimony) introduced errors into the analysis. Cloning techniques were also used to build a DNA library to evaluate the utility of microsatellites for future studies of the population genetics of seamount corals. The studies are being done in close collaboration with Dr. Ted Bayer (cnidarian expert at the Smithsonian Institution), Dr. Scott France (College of Charleston) and Dr. Andrew McArthur (Marine Biology Laboratory).

Tasks Completed:

Representative species were obtained from over 50 anthozoan families that were directly relevant to this phylogenetic study. Many of these specimens were collected during two sets of Pisces V submersible cruises to Hawaiian seamounts through the NOAA Hawaiian Underwater Research Laboratory. Others were collected in shallow water or obtained from museum collections. Full sequences of the 18S rRNA gene have been generated for all specimens. A procedure involving the development of octocoral-specific primers and cloning of three separate fragments of the 18S rRNA gene has been devised to sequence the problematic museum specimens; this procedure has been submitted for publication (Berntson and France, *Mol. Mar. Bio. Biotech.*).

Scientific Results:

Analyses of these anthozoan sequences do not support the traditional taxonomic classification of the octocorals at the ordinal and subordinal levels (question 1). In a few cases where confamilial species have been sequenced, some of the families appear to be monophyletic but others do not. The Order Pennatulacea, which was previously

considered a well-supported group morphologically, is also proving to be polyphyletic. The morphological features most informative for phylogenetic reconstruction appear to be the musculature, nematocysts and overall colony morphology (e.g., dimorphism) of these groups. These observations are critical for evaluating the evolutionary relevance of family-level and higher groupings within the Octocorallia (Berntson and Bayer, submitted to *Mar. Biol.*)

Results from the subclass-level analyses (question 2) show that the two orders within the Subclass Ceriantipatharia are not monophyletic. The antipatharians cluster with the hexacorals, as was suggested by the traditional two-subclass taxonomic system (France et al., 1996, *Mol. Mar. Bio. Biotech*), but the cerianthids branch most ancestrally to all of the remaining anthozoans (Berntson et al., submitted to *Mol. Phylogen. Evol.*).

Support from this award also allowed us to finalize our results from a prior ONR-supported project on dispersal of larvae near Fieberling Guyot (part of the Abrupt Topography ARI). We found that circulation near the seamount did appear to retain larvae, although through a process much different than the one originally proposed (Mullineaux and Mills, 1997, *Deep-Sea Res.*).

Significance:

These new observations on genetic relationships among species within the Class Anthozoa will bring about a substantial revision of the taxonomy of this group at the subclass and ordinal levels. In doing so, this project will change our understanding of the evolution of this group, whose members include major components of the faunas in many patchy, isolated habitats, including seamounts and coral reefs. These observations also have direct relevance for a wide spectrum of other studies:

- 1) Dispersal - The DNA sequences generated in this project provide us with species-specific molecular "tags" which can be developed into larval probes or other methods for identifying morphologically-indistinct anthozoan larvae collected in the field.
- 2) Biodiversity and gene flow - The extraction and sequencing technique developed for preserved museum specimens opens up an enormous wealth of cnidarian specimens that can now be used for studies of biogeography and gene flow. The preliminary results on microsatellites provides a springboard for future studies of the population genetics of seamount corals and other anthozoans.
- 2) Environmental impact - Understanding the diversity and evolution of the Anthozoa will assist us in predicting the susceptibility of important marine communities (e.g., seamount faunas, coral reefs) to anthropogenic impact, and evaluating their resiliency in previously impacted areas.

Publications:

- Berntson, E.A. and S. C. France (submitted). Generating DNA sequence information from museum collections of cnidarian specimens (Phylum Cnidaria: Class Anthozoa). *Molecular Marine Biology and Biotechnology*.
- Berntson, E. A., S. C. France and L. S. Mullineaux (submitted). Phylogenetic relationships within the Class Anthozoa (Phylum Cnidaria) based on 18S ribosomal DNA sequence information. *Molecular Phylogenetics and Evolution*.
- Berntson, E. A. and F. M. Bayer, (for submission Oct. 1998). Genetic variation within the Subclass Octocorallia (Cnidaria: Anthozoa) based on 18S ribosomal DNA. *Marine Biology*.
- Mullineaux, L.S. and S.W. Mills. 1997. A test of the larval retention hypothesis in seamount-generated flows. *Deep-Sea Research* 44: 745-770.
- France, S.C., P.E. Rosel, J.W. Agenbroad, L.S. Mullineaux, T.D. Kocher. 1996. DNA sequence variation of mitochondrial large-subunit rRNA provides support for a two subclass organization of the Anthozoa (Cnidaria). *Molecular Marine Biology and Biotechnology* 5:15-28.
- France, S. C., E. A. Berntson and R. Etter. Diversity and distribution of octocorals and other species on seamounts near Hawaii." In preparation for *Deep-Sea Research*.

Presentations:

- Berntson, E., "Phylogenetic Relationships Within the Anthozoa (Phylum Cnidaria) As Inferred From 18S Ribosomal DNA", presented at the Evolution Meetings held in Boulder, CO (June, 1997)
- Agenbroad*, J.E., S.C. France, L.S. Mullineaux. 1995. Sequence variation in mitochondrial 16S rRNA of three subclasses of anthozoans (Phylum Cnidaria). Poster presented at Keystone Symposium in Santa Fe: Molecular Approaches to Marine Ecology and Evolution. (*J. Ewann Agenbroad changed her name to Ewann Agenbroad Berntson in 1997)

Awards, Honors

- MIT/WHOI Joint Program PhD degree granted to E. Berntson (June 1998)
- Appointment of S. France as Assistant Professor at College of Charleston (Sept. 1997)
- Promotion of L. Mullineaux to Associate Scientist with Tenure (April 1996); appointment to InterRIDGE Steering Committee (1995 to present), and to NSF LARVE Coordinating Committee (1995-1997)

Summary:

The specific goal of this AASERT project is to use molecular techniques to investigate phylogenetic questions within the anthozoans (phylum Cnidaria). The study focuses on current controversies over the ordinal divisions within the Subclass Octocorallia, and the subclass divisions within the Class Anthozoa. Full sequences of the 18S rRNA gene have been generated for over 50 specimens collected fresh or obtained preserved from museum collections. Analyses of octocoral sequences do not support the traditional taxonomic classification of this group at the ordinal and subordinal levels. The order Pennatulacea, which was previously considered a well-supported group morphologically, is proving to be polyphyletic, as are some of the families. Results from the subclass-level analyses show that the two orders within the subclass Ceriantipatharia are not monophyletic. These new observations on genetic relationships among species within the class Anthozoa will bring about a substantial revision of the taxonomy of this group at the sub-class and ordinal levels. In doing so, this project changes our understanding of the evolution of this group, whose members include major components of the faunas in many patchy, isolated habitats, including seamounts and coral reefs.

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